Micropatterning Directional Extracellular Matrix Cues in Hydrogel-based Scaffolds for Cardiac Tissue Engineering
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Statement of Purpose: Cardiac tissue engineering relies on the design of bio-inspired scaffolds to induce cardiomyocytes (CMs) to form functional cardiac tissues in vitro. In particular, there is great potential for scaffolds that recapitulate the structure and composition of the complex extracellular environments observed in the embryonic heart. For example, the embryonic extracellular matrix (ECM) is composed of a fibronectin (FN)-rich basement membrane surrounding highly aligned CMs within a collagen I (COL1)-rich interstitium[1]. However, it is currently challenging to integrate spatially well-defined microscale ECM cues (e.g. FN structures) into 3D scaffolds (e.g. COL1 hydrogel), thus limiting the extent to which the native ECM can be mimicked. Here we present a new scaffold fabrication technique to enable the combination of directional ECM cues into hydrogel-based scaffolds. It relies on standard microcontact printing and allows the implementation of new potent designs for biomimetic scaffolds.

Methods: We modified a previously reported surface-initiated assembly technique[2] in order to transfer 2D patterns of ECM proteins onto hydrogels. Briefly, 20 μm wide lines of fibronectin (FN) were microcontact printed onto a thermally sensitive poly(N-isopropylacrylamide) (PIPAAm)-coated coverslip. The patterned PIPAAm coverslip was then put on a gelatin gel and the PIPAAm dissolved with 20°C water, releasing the FN lines on top of the gelatin. Finally, the patterned gelatin was cut and melted at 37°C over a hydrogel consisting of collagen type I (COL1) or fibrin with or without Matrigel. The quality of the ECM pattern was assessed using fluorescently tagged FN and confocal microscopy. Next, we seeded chick primary CMs onto hydrogels composed of varied concentration of Matrigel, Fibrin, and COL1. We quantified actin alignment after 4 days using immunostaining, confocal microscopy and MATLAB-based image analysis.

Results: We were able to accurately transfer well-defined FN lines onto COL1 and fibrin hydrogels (Fig 1A). FN line width was 19.83 and 19.98 μm on COL1 and fibrin respectively vs. 20.63 μm on PIPAAm before transfer, indicating good pattern fidelity (Fig 1B). CMs cultured on patterned COL1 hydrogels developed distinct sarcomeres after 4 days (Fig 1C). They exhibited spontaneous contraction and strong alignment along the FN lines. Overall, Matrigel dramatically decreased alignment along the FN lines on COL1 based hydrogels (Fig 1D). Alignment on fibrin-based hydrogels was low for all concentrations of fibrinogen and Matrigel (orientational order parameter < 0.5). Thus, we identified high concentration COL1 hydrogels as the ideal substrate to achieve alignment comparable with CMs on 2D PDMS substrates with identical FN lines. Moreover, this showed that FN bioactivity was preserved through transfer to the hydrogels and that it can guide CMs to form anisotropic contractile cardiac sheets.

Figure 1. Micropatterned ECM scaffolds. (A) FN lines (20 μm wide by 20 μm spacing) were microcontact printed and accurately transferred to fibrin (top) and collagen I (bottom) hydrogels. Scale bars = 200 μm. (B) Line width after transfer was within 5% of the original line width. (C) Primary chick CMs seeded on COL1/FN scaffolds were aligned and formed distinct sarcomeres. Samples were stained to show nuclei (blue), α-actinin (red) and actin (green). Scale bar = 50 μm. (D) Alignment (Orientational Order Parameter) was highest on COL1 substrate without Matrigel (n=4). * = p<0.05.

Conclusions: We describe a method to encode directional, microscale ECM cues into hydrogel scaffolds and successfully use it to guide alignment of cardiac cell sheets. As surface-initiated assembly is also compatible with laminin, collagen type IV and collagen type I, we plan to design biomimetic scaffolds with multicomponent ECM patterns inspired by the native extracellular environment. The new tools in this study enable a more systematic and comprehensive approach at engineering fully functional tissues by recapitulating ECM fibril alignment as observed in the intact myocardium within a fully ECM-based hydrogel scaffold.

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References: